

IN THE U.S. PATENT AND TRADEMARK OFFICE

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In re application of
Mello et al.

TECH CENTER 1600/2900

Serial No. 09/490,291
Filing Date: January 20, 2000

Examiner: Schnizer, H.
Group Art Unit: 1653

For: "Methods for the Purification and Aqueous Fiber Spinning of Spider Silks and
other Structural Proteins"

Commissioner of Patents and Trademarks
Attn: Box AMENDMENTS
Washington, D.C. 20231

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OFFICE OF PETITIONS

DECLARATION
(37 CFR § 1.132)

Dear Sir:

Steven Arcidiacono, co-inventor of the above captioned patent application states:

1. My name is Steven Arcidiacono. I am employed as a Research Microbiologist at the U.S. Army Natick Soldier Center, Materials Science Team, Natick, MA 01760 ("NATICK"). I have been employed since 1985.

2. I am making this Declaration in support of an Amendment which I filed on November 12, 2002 in response to the Office Action dated May 31, 2002. I have read and am familiar with the contents of Lombari et al., U.S. Patent No. 5,245,012.

3. The use of HCl as stated in Lombari et al. would be detrimental to the successful practice of the claimed invention. Lombari et al. uses a 50:50 mixture of boiling propionic and HCl, which is not suitable for the recovery of recombinant silk proteins. The treatment of a bacterial pellet

with a solution containing HCl results in the degradation of the recombinant silk protein. The Lombardi et al. method works only when 6N HCl is present and at boiling temperature. This differs with the method of the claimed invention, which is carried out without the presence of HCl and at ambient temperature. Attached hereto is an Appendix which provides information in support of this Declaration.

Declaration

I hereby declare that all statements made herein of my own knowledge are true and that all statement made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and hat such willful false statement may jeopardize the validity of the application of any patent issuing thereon.

11-4-02
Date

Steven Arcidiacono
STEVEN ARCIDIACONO
Co-inventor

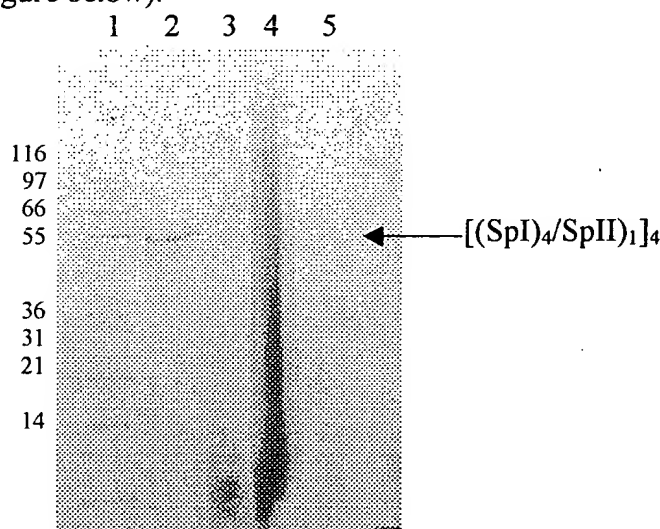
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APPENDIX

Patent response

The examiner states that the present claims are not limited to a method that does not involve affinity chromatography. While this is true, in addition to affinity chromatography silk proteins have also been purified by ion exchange chromatography and could conceivably be purified by any mode of chromatography. The use of chromatography for protein purification in general is not new or unique. Affinity chromatography has been commonly used to purify recombinant silk proteins from bacteria (Prince, McGrath et al. 1995; Lewis, Hinman et al. 1996; Fahnestock and Irwin 1997; Arcidiacono, Mello et al. 1998) lysed by methods other than the 50:50 organic acid:HCl mixture used by Lombardi et al. In fact, affinity chromatography is one of the most common purification approach for all recombinant proteins.

The examiner also states that the Applicants have not submitted any evidence that the addition of other acids (i.e. HCl) would materially change the characteristics of the applicant's invention. We have demonstrated that the use of 2.3N HCl alone or in 50:50 propionic:HCl solution is not suitable for cell lysis because the recombinant silk protein is degraded (see figure below).



This figure depicts an SDS-PAGE gel of recombinant silk protein $[(\text{SpI})_4/\text{SpII})_1]_4$ isolated from a bacterial cell pellet by various acid treatments. Lane 1, Molecular weight markers in kDa; lane 2, 2.3N propionic acid (the method disclosed by the Applicant-not believed to be prior art); lane 3, 2.3N HCl; lane 4, 50:50 propionic/HCl (final concentration 6.7N propionic/6N HCl; lane 5, bacterial cell pellet treated with propionic acid to isolate $[(\text{SpI})_4/\text{SpII})_1]_4$ (as in lane 1) and then treated with 50:50 propionic/HCl (Lombardi et al- the prior art).

When HCl is used in place of an organic acid (i.e. diluted to 2.3N for cell lysis) or in conjunction with an organic acid no recombinant silk protein is present. Use of the 50:50 propionic:HCl mixture results in degradation of all proteins in the lysate, including the recombinant silk. As used in Lombardi et al, the 50:50 mixture is designed not to purify silk but to hydrolyze it, a step required for amino acid composition analysis.

APPENDIX (CONT'D)

The intended use of the applicant's method is not only for isolation of silk protein, but also for processing the protein solution into fibers, films, gels and coatings. This application comprises two distinct novel procedures (1) for the purification of recombinant proteins and (2) the processing of purified silk solutions, purified in any manner, to yield insoluble fibers, films, gels and coatings. The prior art is not capable of performing the applicant's intended use, since the resulting silk in solution is degraded into an unusable form.

Arcidiacono, S., C. Mello, et al. (1998). "Purification and characterization of recombinant spider silk expressed in *Escherichia coli*." Applied Microbiology and Biotechnology **49**: 31-38.

Fahnestock, S. R. and S. L. Irwin (1997). "Synthetic spider dragline silk proteins and their production in *Escherichia coli*." Applied Microbiology and Biotechnology **47**: 23-32.

Lewis, R. V., M. Hinman, et al. (1996). "Expression and purification of spider silk protein: A new strategy for producing repetitive proteins." Protein Expression and Purification **7**: 400-406.

Prince, J. T., K. P. McGrath, et al. (1995). "Construction, cloning, and expression of synthetic genes encoding spider dragline silk." Biochemistry **34**: 10879-10885.